

Genetic Enhancement in *Beta* for Disease Resistance Using Wild Relatives: A Strong Case for the Value of Genetic Conservation¹

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Wild relatives of our present crop plants, although agronomically undesirable, may have acquired many desirable stress-resistant characteristics as a result of their long exposure to nature's stresses. Early U.S. collection activities for wild forms of Beta were conducted by George H. Coons (USDA-ARS) in 1925 and 1935. These collections were mainly wild forms of the section Beta, with major emphasis on leaf spot (Cercospora beticola) resistance. Little was done with this collection until 1976, when John McFarlane (USDA-ARS) transferred it to Salinas, California, to regenerate seed for preservation. Unfortunately, about half of the collection had lost germinability. Immunity to Rhizomania, a devastating root disease discovered in California in 1983, was discovered in several accessions of the Coons' collection by E. D. Whitney. Interestingly, these same accessions subsequently have been found to exhibit Erwinia root rot resistance, sugar beet root maggot tolerance, and moderate leaf spot resistance. The value of wild germplasm is not always apparent immediately. Needs change and the value of wild germplasm may not be realized for years.

Genetische Verbesserung in Beta fuer Krankheitswiderstandsfahigkeit mit dem Gebrauch von wilden Verwandten: Ein wichtiger Punkt fuer den Wert genetischer Erhaltung. Wilde Verwandte unserer gegenwaertigen Kulturpflanzen, obwohl landwirtschaftlich unerwuenscht, moegen viele erwuenschte Stress-widerstandsfahige Eigenschaften angenommen haben, als eine Folge ihres langen Ausgesetztseins gegen die Stresse der Natur. Fruehe U.S. Sammlungen von wilden Beta Formen wurden von George H. Coons (USDA-ARS) in 1925 und 1935 durchgefuehrt. Diese Sammlungen waren hauptsaechlich wilde Formen der Abteilung Beta mit Hauptbetonung auf Blattfleck Widerstandsfahigkeit (Cercospora beticola). Wenig wurde mit dieser Sammlung bis 1976 getan, wo John McFarlane (USDA-ARS) sie nach Salinas, CA ueberwies, um Saatgut fuer die Erhaltung zu erneuern. Leider hatte die Haelfte der Sammlung ihre Keimfahigkeit verloren. Unempfaenglichkeit gegen Rhizomania, eine verheerende Wurzelkrankheit, 1983 in Californien entdeckt, wurde in mehreren Anschaffungen der Coons' Sammlung von E. D. Whitney entdeckt. Interessanterweise zeigten sich anschliessend in diesen gleichen Anschaffungen Erwinia Wurzelsfaele Widerstandsfahigkeit, Zuckerrueben Wurzeln Made Toleranz und gemaessigte Blattfleckenkrankheit Widerstandsfahigkeit. Der Wert der wilden Zellenplasma ist nicht immer sofort sichtbar. Beduerfnisse aendern sich, un der Wert der wilden Zellenplasma mag fuer Jahre noch nicht erkannt sein.

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Wild relatives of our present crop plants are the products of nature and can provide important genes to modern crop plants. Because wild relatives have been exposed to nature's stresses for centuries, they have experienced natural selection for resistance or tolerance for such stresses as heat, frost, drought, flooding, saline or alkaline soils, diseases, and insects. Although these wild relatives generally are undesirable and offer little usable food and fiber value compared to our present cultivated crop plants, they may have developed many desirable stress-resistant characteristics through natural selection.

It was recognized early that genes for specific stress-related traits existed in wild relatives and only a keen eye was needed to identify many of these genes. The identification and transfer of desirable genes into commercial germplasm has had a tremendous impact on the production of important crop plants.

Much of the early work was in the area of disease resistance. As the need for resistance to diseases arose, plant geneticists would survey wild populations for desirable genes. Early collection expeditions were designed primarily to find genes for specific disease resistances. Although productive, little attention was paid to preserving the germplasm.

Other desirable characters soon were recognized in this wild germplasm and thus the need for preservation was illustrated. Since wild relatives generally are considered weeds and their native habitats are being destroyed in many areas of the world, gene banks have been established to preserve much of this native germplasm. Although valuable traits may not be apparent in the wild germplasm, future needs cannot be anticipated. Germplasm of no apparent economic value today may someday prove to be extremely valuable genetic material.

LITERATURE REVIEW

Cultivated beets belong to the genus *Beta* (Chenopodiaceae). The Near East is considered the center of origin of *Beta*. Wild forms are found in China, India, central and European Asia, along the Mediterranean coasts of Europe and Africa, and the European shores of the Atlantic Ocean. *Beta* generally is divided into three sections and 10 species (Zossimovitch 1940). The Section *Beta* is by far the most important section. From it come all the cultivated and economic forms of beet (sugar beet, red garden beet, leaf vegetable beet, swiss chard, and fodder beet). Although only one species (*B. vulgaris* L.) generally is considered as representing this section, some authorities have divided it into the following taxa: *B. vulgaris*, *B. maritima* L., *B. macrocarpa* Guss., and *B. atriplicifolia* Roug (Coons 1975). These cross readily with each other and appear to be homologous. The last three taxa, wild forms of the cultivated *B. vulgaris*, are found growing in their native habitat along the coasts of the Mediterranean Sea and European shores of the Atlantic Ocean. A recent collection expedition to southern Italy documented the erosion that was taking place in native populations of *B. maritima*. "Intensive farming practices have forced the surviving native flora to fence lines and roadsides. The practice of cutting and burning roadsides and fence lines along with the increased tourist activities is gradually eliminating these native populations" (Doney 1985).

The first historical documentation of *Beta* germplasm being collected was by Munerati (Biancardi and Biaggi 1979). The first U.S. efforts to collect germplasm

of wild forms of *Beta* were conducted by George H. Coons (USDA-ARS) in 1925 (Coons 1975). He collected seed of *B. maritima* along the coasts of southwestern France, the southeastern coast of England, and the coast of Italy near the mouth of the Po River. His chief purpose was to obtain resistance to leaf spot (*Cercospora beticola*). His collections were crossed to *B. vulgaris* (sugar beet) and tested for leaf spot resistance. The results were rather disappointing and resulted in little attention being given to the collection. "The reactions of the hybrids were not impressive, and it is clear now that they were not adequately studied in the later generations. Also, some colonies of wild beets were free from beet rust. . . Inasmuch as this disease is often of minor importance in the U.S.A., I did not try to note it or collect samples" (Coons 1975).

Coons conducted another collection expedition in 1935 to England, France, Spain, Portugal, the Madeira Islands, the Canary Islands, Italy, Greece, and Turkey (Coons 1975). The major emphasis of this expedition was to collect germplasm of most of the *Beta* species. The expedition was successful and seed samples of most *Beta* species were obtained. Limited evaluation of this germplasm suggested it to be of little use. "I found a few colonies of *B. maritima* in France and England that were free from leaf spot. Plants grown from this seed were crossed with individual plants of sugarbeet, and the hybrids were tested repeatedly. Resistance was not notably improved, and the tests were given up, probably too soon" (Coons 1975).

In 1969, Dewey Stewart (USDA-ARS) revived the idea that, to make continued progress in leaf spot resistance, it would be necessary to go back to *B. maritima* for new genes. Stewart found colonies of *B. maritima* free of *Cercospora* leaf spot from Wembury Bay, England, Kilmore Quay, Ireland, and Kalundborg, Denmark. These accessions showed moderate resistance to leaf spot at Beltsville, Maryland. By this time, some degree of resistance to leaf spot had been found in existing commercial stocks of sugar beet and the transfer of resistance from *B. maritima* to commercial stocks was abandoned (Coons 1975).

The Coons and Stewart collections were left with G. E. Coe in Beltsville, who continued the evaluation of this germplasm on a very limited scale. Efforts were made by Coons, Stewart, and Coe to increase this material, but time and adequate storage facilities were not available. John McFarlane recognized the need to maintain and preserve this germplasm and in 1976 transferred most of the collections to Salinas, California, for seed increase. Unfortunately, seed storage facilities were lacking at Beltsville and a number of accessions failed to germinate and were lost. Those that germinated were increased under controlled isolated conditions and the seed increase deposited in the NC-7 Plant Introduction Station at Ames, Iowa.

Rhizomania, a root disease not previously found in the U.S., was discovered in sugar beet fields near Paso Robles, California, in 1983 (Duffus et al. 1984). A year later, Rhizomania had been identified in 71 fields containing more than 2400 ha and in other sugar beet production areas of the state. Rhizomania is caused by a virus known as beet necrotic yellow vein, which is vectored by and preserved in a fungus, *Polymyxa betae*. The disease has reached epidemic proportions in recent years in many sugar beet growing areas of Europe and Japan. Typically, Rhizomania causes plant losses of 20–50% in infested fields, can cause total crop failure, and often reduces sugar yield by as much as 25% (Whitney 1986). Shortly after the discovery of Rhizomania in California, E. D. Whitney, a plant pathologist

(USDA-ARS, Salinas, California), began screening for Rhizomania resistance (Whitney 1986). Among the germplasm screened were the Coons and Stewart collections that McFarlane had salvaged and increased. A number of the same accessions from the Coons and Stewart collections were tested also for sugar beet root maggot resistance and Cercospora leaf spot resistance. The sugar beet root maggot (*Tetanops myopaeformis*), a native fly, has become a major pest of sugar beets in approximately two-thirds of the U.S. acreage. In 1983 the Sugarbeet Crop Advisory Committee identified sugar beet root maggot resistance as a top research priority. Cercospora leaf spot has been an important disease of sugar beets for many years, annually infecting up to half of the U.S. acreage and causing millions of dollars of loss each year.

METHODS

Accessions tested were biennial or semi-biennial survivors of the Coons and Stewart collections. Screening for Rhizomania resistance was conducted in the greenhouse as previously described (Whitney 1989). Disease index ratings were made on plants (2 mo old) grown in Rhizomania infested soil. The rating scale was from 0 to 9, with 0 = no symptoms and 9 = plants dead. Root sap of plants with low disease ratings was tested for the presence of beet necrotic yellow vein virus (BNYVV) by the enzyme-linked immunosorbent assay (ELISA) (Clark and Adams 1977).

Erwinia resistance testing was conducted on greenhouse plants grown in Erwinia infested soil as described (Whitney and Lewellen 1977, 1978). Ratings were based on a scale of 0 to 9, with 0 = no visible symptoms and 9 = plants dead.

Damage ratings for root maggot resistance were made in field trials conducted at St. Thomas, North Dakota, in 1986. The field trial site was selected based on an extremely high natural infestation. Because of seed quantity, the trial was planted as an augmented randomized block design with three and four replications. Damage was rated on a scale of 0 to 5, with 0 = no damage and 5 = severe damage (Fay 1986).

Accessions were evaluated for Cercospora leaf spot resistance by Betaseed Inc., at Shakopee, Minnesota. Methods of evaluation were standard procedures for leaf spot evaluation (Smith and Gaskill 1970). Ratings were based on a scale of 1 to 9, with 1 = no visible infection and 9 = severely infected.

RESULTS

Several of the accessions exhibited no symptoms of Rhizomania infection and tested negative for the presence of BNYVV virus by the ELISA assay (Table 1). Interestingly, some of the accessions exhibiting resistance to Rhizomania also showed resistance to Erwinia root rot, i.e., WB151, WB177, WB178, and WB187. Some of these accessions have been further evaluated and found to carry a dominant gene for resistance to Rhizomania (Whitney 1986). Work is currently under way to transfer this resistance to commercial sugar beet germplasm (Lewellen et al. 1987).

Not as many accessions were screened for sugar beet root maggot and Cercospora leaf spot resistance as were for Rhizomania. However, some of the same accessions showed a tolerance to sugar beet root maggot and a moderate resistance to leaf spot when compared to a standard check hybrid (Table 2).

Table 1. Rhizomania and Erwinia disease index ratings for a number of wild *B. maritima* accessions. Ratings conducted by E. D. Whitney, USDA-ARS, Salinas, California.

<i>B. maritima</i> accession	Rhizomania		ELISA (A 405 nm)	Erwinia disease index ^a
	Source	Disease index ^a		
WB 41	Denmark	0.4d ^b	0.162	1.25
WB 42	Denmark	0.9cd	0.030	1.25
WB 54	France	6.6a		1.25
WB 69	?	4.2b		0.00
WB 71	?	4.5b		3.15
WB 73	?	3.1bc		0.65
WB 151	Denmark	0.5d	0.013	0.00
WB 173	England	4.0b		0.00
WB 177	Denmark	1.9cd	0.124	0.00
WB 178	England	0.9cd		0.00
WB 179	England	1.7cd	0.248	1.90
WB 180	Denmark	1.9cd	0.317	0.00
WB 181	Ireland	-		0.65
WB 182	England	2.5bcd		0.00
WB 184	England	3.0bc	0.534	2.50
WB 185	England	4.0b		0.00
WB 187	England	1.6cd	0.249	0.00
WB 190	England	1.3cd	0.474	0.00
WB 191	Denmark	2.6bcd	0.012	0.00
WB 257	Italy	4.6b		0.65
WB 318	France	0.8d	0.564	0.00
8717		2.6bcd	0.413	
Sugar Beet Check				

^a Disease index based on a rating of 0 to 9 with 0 = no symptoms and 9 = dead.

^b Means followed by a common letter are not significantly different at $P = 0.05$ by Duncan's multiple range test.

The rather astonishing discovery was that some of these accessions contained excellent to moderate resistance to all four pests (note WB42 and WB151, Tables 1 and 2). It is not known if these multiple resistances are linked or pleiotropically associated.

If these resistances can be satisfactorily transferred, the original germplasm that was thought to be of little value and was almost lost may prove to be of tremendous value. It is doubtful if Dr. Coons realized that the germplasm he collected in 1925 and 1935, which had twisted, sprangled roots and leathery small leaves, may turn out to be an important source of disease resistance.

The value of wild germplasm is not always apparent in its native habitat or after its initial evaluation. Needs change, and what may be important today may be of little value 20 yr from now and vice versa. As our ability to identify and transfer specific genetic traits improves, wild germplasm can be utilized more effectively.

Because of the continued loss of wild germplasm in its native habitat, preser-

Table 2. Resistance and/or damage ratings for *Cercospora* leaf spot (mean of 1984 and 1985 tests) and sugarbeet root maggot (1986) of several wild *B. maritima* accessions. Ratings conducted by Betaseed, Inc., Shakopee, Minnesota, for leaf spot and by Deborah H. Fay, North Dakota State University at St. Thomas, North Dakota, for root maggot (Fay 1986).

<i>B. maritima</i> accession	Source	Sugarbeet root maggot		<i>Cercospora</i> ratings ^b
		Damage rating ^a	% Plant mortality	
WB185	England	2.80a	2a ^c	3.3
WB151	Denmark	3.10ab	2a	3.5
WB42	Denmark	3.10ab	8ab	3.2
WB178	England	3.13ab	17abcd	-
WB182	England	3.58bcd	13abc	3.1
WB179	England	3.74cde	16abcd	3.6
WB180	Denmark	3.78cde	25bcd	3.5
WB65	France	4.15e	35d	5.3
Standard (commercial hybrid)		4.23e	61e	4.6
LSD 0.05				0.4

^a Damage rating scale of 0 to 5 with 0 = no damage and 5 = severely damaged.

^b Rating scale of 1 to 9 with 1 = no infection and 9 = severely infected.

^c Means followed by a common letter are not significantly different at $P = 0.05$ by Duncan's multiple range test.

vation of germplasm for future generations is imperative. Its value may not be realized for many years. Its preservation, therefore, serves as an insurance for future food and fiber needs.

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Methods and Approaches in Ethnobotany. S. K. Jain [ed.]. Society of Ethnobotanists, Lucknow, India. 1989. Rs. 80, U.S. \$20.00. Copies available from Society of Ethnobotanists, Central Drug Research Institute, Lucknow 226001, India.

Dr. S. K. Jain, outstanding ethnobotanist of India, has edited this highly practical book in which 18 leaders of the fast-developing field of research in India have contributed. There is much in this small volume that is immediately pertinent to ethnobotany anywhere in the world. It can enthusiastically be recommended to all who are directly or tangentially interested in this discipline.

The volume has nineteen contributions: Ethnobotany, a holistic approach to man/plant relationships; Basic considerations in ethnobotanical methods and techniques; Biological screening of plants; Studies in Meghalaya, herbal medicines; Global perspective on plant domestication; Ethnobotany in art and literature; Human physiology and nutrition; Therapeutic terms in ethnobotany; Dravyaguna—the science of properties and actions of drugs; Ayurveda approaches in evolution of drugs; Phytochemistry; Ethnobotanists commemorated in generic names; Economic development of backward people; The message of ethnobotany for the next century; Ethnobotany and other sciences; Ethnobotany of Kumaon Himalayas; Three primitive tribes of Central India; Archaeological monuments and sites; The role of pharmacology. There follows an appendix: Report on the training course and workshop.

It is the wide scope of coverage discussed by so many men and women active in ethnobotanical fields that sets this book apart from most volumes dedicated to the history of ethnobotany and its modern role. It is, in short, a model for anyone working in ethnobotany and a credit to the extraordinary ethnobotanical activity of India.

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